Cat. # RR182A

For Research Use

TaKaRa

Bacterial 16S rDNA PCR Kit Fast (800)

Product Manual

v202203Da

Table of Contents

I. Description

Microorganisms are typically identified by morphological, physiological, and biochemical characteristics, among other attributes. However, procedures for characterizing these features are time-consuming and sometimes yield incorrect or no results.
DNA analysis has been increasingly used for microorganism identification. Ribosomal DNA (rDNA) is often analyzed for this purpose, as it is a suitable size and contains variable regions that can be used to discriminate species.
The Bacterial 16S rDNA PCR Kit Fast (800) is designed to amplify a specific region (approx. 0.8 kb) within bacterial 16S rDNA, and includes primers for sequencing the resulting PCR products. The PCR enzyme included with the kit is a modified *Taq* DNA polymerase, which allows rapid PCR amplification. Additionally, this hot-start enzyme is formulated with an anti-*Taq* antibody, which prevents nonspecific amplification that results from pre-cycle mispriming or primer-dimer formation. The sequencing information obtained with this kit can be compared with sequence databases to classify microorganisms based on homology.

* The analytical procedure used with the primers provided in this kit is published in the section titled "Rapid Identification of Microorganisms Using Genetic Analysis" under General Information in the 18th Edition of the Japanese Pharmacopoeia.

NOTE: This kit may not be suitable for some unidentified bacterial species.
To identify fungi, use Fungal rDNA (ITS1) PCR Kit Fast (Cat. #RR183A) or Fungal rDNA (D1/D2) PCR Kit Fast (Cat. #RR184A).

II. Components (25 µl PCR reaction volume, 50 reactions)

1) *TaKaRa Taq*™ HS Fast Detect Premix	2X	625 µl
2) 16S rDNA Primer Mix (800)*1	10X	125 µl
3) dH_2O		650 µl
4) Positive Control (*E. coli* DNA)	1 ng/µl	25 µl
5) Sequencing Primer 10F	7.5 pmol/µl	50 µl
6) Sequencing Primer 800R	7.5 pmol/µl	50 µl

*1 The 16S rDNA Primer Mix (800) includes primers that are identical in sequence to Sequencing Primer 10F and Sequencing Primer 800R.

III. Materials Required but not Provided

- PCR thermal cycler
  TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)*2
  TaKaRa PCR Thermal Cycler Dice Gradient (Cat. #TP600)*2
- PCR tubes*3
  0.2 ml Hi-Tube Dome Cap (Cat. #NJ200)
  0.2 ml Hi-Tube Flat Cap Recovery (Cat. #NJ203: discontinued)
  0.2 ml Hi-8-Tube (Cat. #NJ300)
  0.2 ml Hi-8-Dome Cap (Cat. #NJ301)
  0.2 ml Hi-8-Flat Cap (Cat. #NJ302)
- Agarose gel electrophoresis apparatus
  Mupid-2plus (Cat. #M-2P)
  Mupid-exU (Cat. #EXU-1)
- Agarose
  Agarose L03 [TAKARA] (Cat. #5003/5003B)
  PrimeGel™ Agarose PCR-Sieve (Cat. #5810A) or a similar product
- DNA staining dye
  Ethidium bromide or a similar product
- Microcentrifuge
- Micropipettes and tips (autoclaved)
- DNA preparation kit
  SimplePrep™ reagent for DNA (Cat. #9180)*2
  NucleoSpin Tissue (Cat. #740952.10/.50/.250)*2 or a similar product
- PCR product purification kit
  NucleoSpin Gel and PCR Clean-up (Cat. #740609.10/.50/.250)*2 or a similar product

*2 Not available in all geographic locations. Check for availability in your area.

*3 For the TaKaRa PCR Thermal Cycler Fast (Cat. #TP450: discontinued), use flat-cap tubes. Dome-cap tubes are not compatible. See the manual of each apparatus for details.

IV. Storage -20℃

Excessive freezing-thawing cycles may decrease the activity of *TaKaRa Taq* HS Fast Detect Premix.
Avoid vigorous mixing when thawing. Gently invert the tube to mix after thawing.

V. Protocol

V-1. Preparation of DNA samples

Prepare DNA samples for PCR in accordance with the method described below from colonies or liquid cultures prepared from individual colonies. For samples that are a mixture of multiple microorganisms, sequence analysis will not provide the correct information as PCR will generate multiple amplification products. In such a case, perform cloning of the amplification products and analyze the sequence of individual clones.
If DNA amplification is not observed using a DNA sample obtained by the procedure described in V-1-1. "Preparation of heat-extracted samples", try one of the procedures described below (V-1-2. through V-1-5.).

V-1-1. Preparation of heat-extracted samples

1. For colonies, pick individual colonies and suspend in 100 μl of sterile distilled water in a microtube.
   For liquid cultures, dispense 50 μl of the culture into a microtube, centrifuge at 8,000*g* for 5 minutes at room temperature, and remove the supernatant. Add 100 μl of sterile distilled water to the cell pellet and resuspend.
2. Heat at 95℃ for 15 minutes.
3. Centrifuge briefly and use the supernatant as the sample DNA solution.

V-1-2. Preparation of alkaline heat-extracted samples

1. For colonies, pick individual colonies and suspend in 50 μl of sterile distilled water in a microtube.
   For liquid cultures, dispense 50 μl of the culture into a microtube, centrifuge at 8,000*g* for 5 minutes at room temperature, and remove the supernatant. Add 50 μl of sterile distilled water to the cell pellet and resuspend.
2. Add 50 μl of 100 mM NaOH to the suspension, mix, and heat treat at 95℃ for 15 minutes.
3. Add 11 μl of 1M Tris-HCl (pH 7.0) and mix.
4. Centrifuge briefly and use the supernatant as the sample DNA solution

V-1-3. Preparation with SimplePrep reagent for DNA (Cat. #9180)

1. For colonies, pick individual colonies and suspend 100 μl of sterile distilled water in a microtube.
   For liquid cultures, dispense 50 μl of the culture into a microtube, centrifuge at 8,000*g* for 5 minutes at room temperature, and remove the supernatant. Add 100 μl of sterile distilled water to the cell pellet and resuspend.
2. In a 0.2-ml PCR tube, mix 20 μl Reagent A and 4 μl Reagent B supplied in the SimplePrep reagent for DNA, add 20 μl of the cell suspension, and incubate in a thermal cycler at 37℃ for 6 minutes, then 95℃ for 3 minutes.
3. Add 80 μl of sterile distilled water, pipette to mix, and use as the sample DNA solution (use at no more than 1/10 of the reaction mixture).

V-1-4. Preparation by bead treatment

Sample DNA solutions can also be prepared using commercially available beads for DNA preparation. The following is an example of DNA sample preparation using EZ-Beads (Sumika Chemical Analysis Service/AMR).

1. For colonies, pick individual colonies and suspend each colony in 100 μl of sterile distilled water in a microtube.
   For liquid cultures, dispense 50 μl of the culture into a microtube, centrifuge at 8,000 X *g* for 5 minutes at room temperature, and remove the supernatant. Add 100 μl of sterile distilled water to the cell pellet and resuspend.
2. Add the cell suspension to a tube containing EZ-Beads.
3. Use a bead mill or a vortex mixer at the maximum speed for 2 minutes.
4. Add 100 μl of sterile distilled water, mix gently, and centrifuge to obtain the supernatant for use as the sample DNA solution.

V-1-5. Preparation with NucleoSpin Tissue (Cat. #740952.10)

Suspend cells from colonies in 200 μl of Buffer T1 or add 200 μl of Buffer T1 to a cell pellet obtained from a liquid culture. Perform extraction according to the product manual.

[NOTE] Use sample DNA solutions as soon as possible after preparation. For short-term storage, store sample DNA solutions at 4°C until use. Store samples that will not be used immediately at –20°C.

V-2. PCR Amplification of the 16S rDNA region

(1) Prepare the following reaction mixture

<Per reaction>

Reagent	Volume
TaKaRa Taq HS Fast Detect Premix (2X)	12.5 μl
16S rDNA Primer Mix (800) (10X)	2.5 μl
Sample DNA solution	1 - 2.5 μl*4
dH_2O	X μl*5
Total	25 μl

*4 Mix all components of the reaction mixture except the sample DNA solution. Dispense the mixture into 0.2 ml PCR tubes and then add the sample DNA solution. Some sample DNA solutions may inhibit PCR depending on the method of sample preparation. In such cases, reduce the amount of sample DNA solution or dilute the sample solution with sterile water.

*5 Depending on the volume of the sample DNA solution used, add an appropriate amount of dH_2O to obtain a final volume of 25 μl.

NOTE: If the amount of PCR product is not sufficient for sequence analysis, perform the reaction in twice the volume.

(2) Dispense the prepared reaction mixture into 0.2-ml tubes.

(3) Add the sample DNA solution and place each tube in a thermal cycler.
When necessary, add dH_2O or 1 μl of Positive Control (*E. coli* DNA) in place of the sample DNA solution to serve as the negative or positive control, respectively.

(4) Perform PCR using the conditions below.

[Conventional thermal cycler]

94℃	5 sec	25 cycles*6
55℃	1 sec	
68℃	4 sec	

[Fast thermal cycler*7]

94℃	5 sec	25 cycles*6
55℃	1 sec	
68℃	8 sec	

[When using other thermal cycler]
This kit is designed to be suitable at fast PCR condition which is set at brief time of each PCR step. When used a thermal cycler other than the instruments of Takara Bio Inc., the PCR condition above may not be used for proper PCR reaction. Try to perform the PCR condition below, if PCR product amplified at the Positive Control reaction is low.

92℃	5 sec	25 cycles*6
50℃	1 sec	
68℃	8 sec	

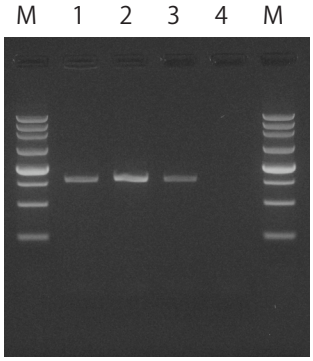
*6 The amount of PCR amplification product may be increased by increasing the cycle number. Doing so, however, may result in the amplification of environmental microbial DNA.

*7 Use TaKaRa PCR Thermal Cycler Fast or a thermal cycler that has a ramp temperature speed of 5℃ or more.

Store at -20℃ if the following analysis will not take place immediately after reaction completion.

V-3. Confirmation of PCR products

After PCR reaction completion, analyze a part of the reaction mixture by agarose gel (e.g., 2% gel) electrophoresis. Make sure that the sample DNA solution and the positive control each yield amplification products of approximately 0.8 kb.



1: *E. coli*, heat-extracted sample
2: *B. subtilis*, alkaline heat-extracted sample
3: Positive Control
4: Negative Control
M: 250 bp DNA Ladder (Dye Plus)

2% agarose

V-4. Purification of PCR amplification products

After confirming amplification products by electrophoresis, purify the PCR products for sequence analysis. If the PCR reaction yields a single band of approximately 0.8 kb, purify the remaining reaction mixture directly using a purification kit such as NucleoSpin Gel and PCR Clean-up (Cat. #740609.10). If nonspecific bands are observed, recover the target band from the agarose gel and purify. NucleoSpin Gel and PCR Clean-up can also be used for the purification of the target band from the agarose gel. Determine the quantity of the purified PCR products by measuring their absorbance at A_{260}.

V-5. Sequence analysis of PCR amplification products

Use Sequencing Primer 10F supplied in this kit for sequencing (single-strand sequencing) the purified PCR products.
Use Sequencing Primer 800R to sequence both strands when necessary. Double-strand sequencing provides high-fidelity sequence data.
Primers are supplied at 7.5 pmol/μl. Please adjust the amount used as appropriate.
Use between 20 and 150 ng of purified PCR product per reaction.

V-6. Analysis of nucleotide sequence

Perform a homology search of databases with the nucleotide sequence obtained by sequencing and deduce the type of microorganism based on the search results.

A BLAST search can be used:
NCBI: https://blast.ncbi.nlm.nih.gov/Blast.cgi
DDBJ: https://www.ddbj.nig.ac.jp/services/blast-e.html

VI. Troubleshooting

No PCR product

- The amount of DNA obtained is not sufficient for PCR amplification.
  → Increase the amount of microorganism used to prepare the DNA sample.
  → Try other procedures for preparing the DNA sample.
- PCR inhibitors are present
  → Dilute the prepared DNA sample as appropriate before use.
- The target microorganism is a fungus.
  → Use a kit for fungal amplification: Fungal rDNA (ITS1) PCR Kit Fast (Cat. #RR183A) or Fungal rDNA (D1/D2) PCR Kit Fast (Cat. #RR184A).

VII. Related Products

Bacterial 16S rDNA PCR Kit (Cat. #RR180A)*8
Fungal rDNA (ITS1) PCR Kit Fast (Cat. #RR183A)*8
Fungal rDNA (D1/D2) PCR Kit Fast (Cat. #RR184A)*8
TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)*8
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)*8
0.2 ml Hi-Tube Dome Cap (Cat. #NJ200)
0.2 ml Hi-8-Tube (Cat. #NJ300)
0.2 ml Hi-8-Dome Cap (Cat. #NJ301)
0.2 ml Hi-8-Flat Cap (Cat. #NJ302)
SimplePrep™ reagent for DNA (Cat. #9180)*8
NucleoSpin Tissue (Cat. #740952.10/.50/.250)*8
NucleoSpin Gel and PCR Clean-up (Cat. #740609.10/.50/.250)*8

*8 Not available in all geographic locations. Check for availability in your area.